IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	S. F. Fabijanski, et al.
Appln. No.	:	Continuation of PCT/CA99/01208
Filed:	:	December 22, 1999
Title	:	Methods and Genetic Compositions to Limit Out-crossing and Undesired Gene Flow in Crop Plants
Grp./A.U.	:	

Honorable Commissioner for Patents
Washington, D.C. 20231

AMENDMENT

Sir:

By way of a Preliminary Amendment, please amend the above-identified application as follows:

In the Specification:

At page 1, after the title, please add the following new paragraph:

--CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of International Application No. PCT/CA99/01208, having an international filing date of December 22, 1999, and which claims priority from United States Provisional Patent Application Serial No. 60/113,545, filed on December 22, 1998, which are both incorporated herein by reference--.

In the Claims:

Please replace claims 1-97 of record with new claims 98-119 attached hereto.

REMARKS

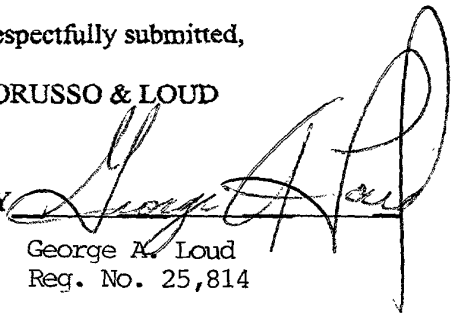
Claims 1-97 of record are cancelled. New claims 98-119 are added.

The claims of the international application contained multiple-dependent claims, which were dependent on other multiple-dependent claims. The new claims are amended to define the invention with greater clarity and to conform the claim dependencies with United States practice. Applicants respectfully submit that the new

claims are fully supported by the original specification and claims and that the amendments therefore do not constitute new matter.

Respectfully submitted,

LORUSSO & LOUD

BY 

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Date: June 22, 2001

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CLAIMS:

98. A method of producing a genetically modified plant, comprising:

(a) providing at least one plant cell capable of being transformed and being generated into a whole plant;

(b) introducing into the at least one plant cell:

(i) a repressible lethal gene encoding a gene product having an activity lethal to plant cells, and

(ii) a repressor gene encoding a gene product capable of repressing the activity of the gene product of the repressible lethal gene;

(c) generating a plurality of whole plants from the at least one plant cell; and

(d) selecting for a genetically modified plant descended from or derived from at least one of the plurality of whole plants by determining incorporation and mutually independent segregation of the repressor gene and the repressible lethal gene within the genetically modified plant.

99. The method of claim 98, wherein said introducing further comprises providing the repressible lethal gene in a first vector construct and providing the repressor gene in a second vector construct, and further comprising crossing at least two plants of the plurality of whole plants prior to said selecting.

100. The method of claim 99, further comprising providing a conditionally lethal gene in the first vector construct, the conditionally lethal gene configured for encoding a gene product lethal to cells of the genetically modified plant upon

application of a chemical or physiological stress to the genetically modified plant.

101. The method of claim 98, wherein said determining mutually independent segregation of the repressor gene and the repressible lethal gene comprises determining that the repressible lethal gene and the repressor gene are located on respective opposite sister chromosomes of a chromosome pair of a plant cell of the genetically modified plant.

102. The method of claim 98, wherein said introducing comprises providing the repressible lethal gene and the repressor gene as part of a single vector construct, and further comprising

introducing at least one of a recombinase or a transposase having recognition specificity to at least one nucleic acid site of the single vector construct, the recombinase or a transposase configured for effecting the mutually independent segregation of the repressor gene and the repressible lethal gene by transposition or recombination of at least one nucleic acid in the single vector construct.

103. The method of claim 98, further comprising providing a tissue-specific promoter in transcriptional control of at least one of the repressible lethal gene or the repressor gene.

104. The method of claim 103, wherein said providing the tissue-specific promoter comprises providing a seed-specific promoter.

105. The method of claim 104, wherein the seed-specific promoter is a phaseolin promoter.

106. The method of claim 98, further comprising providing an inducible promoter in transcriptional control of the repressor gene.

107. The method of claim 98, further comprising providing a DNA operator sequence in operable association with the repressible lethal gene, the DNA operator sequence adapted for binding the gene product of the repressor gene to repress transcription of the gene product of the repressible lethal gene.

108. The method of claim 98, wherein the activity of the gene product of the repressible lethal gene comprises over-expression or under-expression of a naturally occurring plant growth regulating substance in a plant cell of the genetically modified plant.

109. The method of claim 98, wherein the repressible lethal gene is selected from the group consisting of oncogenes 1 and 2, oncogene 4 of *Agrobacterium*, a gene encoding a ribosome inactivating protein, a gene encoding diptheria A chain toxin, and a gene encoding a ribonuclease.

110. The method of claim 98, further comprising linking a gene encoding a trait of interest with the repressible lethal gene in a first vector construct, and wherein said introducing comprises introducing the first vector construct to the at least one plant cell.

111. The method of claim 98, wherein the repressor comprises a repressor selected from the group consisting of an antisense RNA, a ribozyme, and a sense gene.

112. The method of claim 98, wherein said generating the plurality of whole plants comprises generating at least one plant which is homozygous for the repressible lethal gene and the repressor gene.

113. The method of claim 112, further comprising crossing the at least one plant which is homozygous for the repressible

lethal gene and the repressor gene with a second plant to produce the genetically modified plant.

114. A method of producing a genetically modified plant having at least one repressible lethal gene expressed during outcrossing or introgression of alien germplasm, comprising:

(a) providing a plant cell capable of being transformed and being regenerated to a whole plant;

(b) introducing into the plant cell:

(i) a first repressible lethal gene under transcriptional control of a seed-specific promoter, the first repressible lethal gene encoding a gene product having a first gene product activity lethal to plant cells;

(ii) a first operator sequence in operable association with the first repressible lethal gene;

(iii) a first bacterial repressor gene, the first bacterial repressor gene encoding a gene product capable of repressing the first gene product activity by binding to the first operator sequence;

(iv) a second repressible lethal gene under transcriptional control of a seed-specific promoter, the second repressible lethal gene encoding a gene product having a second gene product activity lethal to said plant cells;

(v) a second operator sequence in operable association with the second repressible lethal gene;

(vi) a second repressor gene, the second repressor gene encoding a gene product capable of repressing the second gene product activity by binding to the second operator sequence;

(vii) at least one gene encoding a trait of interest linked to at least one of the first and second repressible lethal genes;

(c) regenerating a whole plant from the plant cell; and

(d) selecting for a genetically modified plant descended from or derived from the whole plant by determining incorporation and mutually independent segregation of the first repressor gene from the first repressible lethal gene, and by determining incorporation and mutually independent segregation of the second repressor gene from the second repressible lethal gene within the genetically modified plant.

115. The method of claim 114, wherein said introducing further comprises providing the first repressible lethal gene, the at least one gene encoding the trait of interest, the first operator sequence and the second repressor gene in a first genetic construct, and providing the second repressible lethal gene, the second operator sequence and the first repressor gene in a second genetic construct.

116. The method of clam 114, wherein said introducing comprises introducing the first repressible lethal gene, the first operator sequence, the at least one gene encoding the trait of interest, the second repressor gene, the second repressible lethal gene, the second operator sequence and the first repressor gene to the plant cell in a single transformation vector.

117. The method of claim 116, further comprising introducing to the plant cell at least one recombinase and/or transposase configured for effecting the mutually independent segregation of the first repressor gene from the first repressible lethal gene and the mutually independent segregation of the second repressor gene from the second repressible lethal gene by

transposition or recombination of at least one nucleic acid in the single transformation vector.

118. A method of producing a genetically modified plant having at least one repressible lethal gene expressed during outcrossing or introgression of alien germplasm, comprising:

(a) providing a plant cell capable of being transformed and being regenerated to a whole plant;

(b) introducing into the plant cell:

(i) a first repressible lethal gene under transcriptional control of a seed-specific promoter, the first repressible lethal gene encoding a gene product having a first gene product activity lethal to plant cells;

(ii) a first operator sequence in operable association with the first repressible lethal gene;

(iii) a first bacterial repressor gene, the first bacterial repressor gene encoding a gene product capable of repressing the first gene product activity by binding to the first operator sequence;

(iv) at least one gene encoding a trait of interest linked to the first repressible lethal gene;

(c) regenerating a whole plant from the plant cell; and

(d) selecting for a genetically modified plant descended from or derived from the whole plant by determining incorporation and mutually independent segregation of the first repressor gene from the first repressible lethal gene.

119. A plant comprising at least one plant cell derived from or descended from a genetically modified plant produced by the method of claim 98, claim 114, or claim 118.

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DOW AGROSCIENCES CANADA INC.
(S. F. Fabijanski, et al)

Serial No. PCT/CA99/01208

METHODS AND GENETIC COMPOSITIONS TO LIMIT OUTCROSSING AND UNDESIRED GENE FLOW IN CROP PLANTS

To: International Preliminary Examining Authority
European Patent Office
D-80298 Munich

Dear Sir:

RESPONSE TO WRITTEN OPINION

AND AMENDMENT PURSUANT TO ARTICLE 34 PCT

In response to the Written Opinion mailed on February 22, 2001, please amend the subject application as follows, without prejudice or disclaimer:

IN THE DESCRIPTION

Please replace pages 65, 66, 67, 68, 70 and 71 of record, with new pages 65, 66, 67, 68, 70 and 71, enclosed herewith.

IN THE CLAIMS

Please replace pages 85-106 of record, with new pages 85-100, enclosed herewith.

IN THE SEQUENCE LISTING

Please replace the Sequence Listing pages of record with new pages 1/3-3/3, enclosed herewith.

REMARKS

The Sequence Listing is amended to correct a typographic error in SEQ ID NO. 8. Nucleotides 29 and 30 should be G and C respectively, not C and G. Support for the amendment is found in the description of record at page 68, in sequence G1P4.

A copy of the amended Sequence Listing in computer-readable form is enclosed. The copy of the Sequence Listing in computer-readable form is the same as the amended Sequence Listing contained in the description.

The description has been amended to identify sequences in bold face and underline font in accordance with the text of the description of record. Support for the amendments is found in the description of record, which describes the portions of the sequences to be illustrated in bold face or underlined text.

There are now 97 claims pending. Claim 10 is amended to clarify that oncogenes 1 and 2 are of *Agrobacterium*. Claim 27 is cancelled. Claims 28-87 are renumbered accordingly as claims 27-86. Claim 57 of record is amended to clarify that the plant seed is derived from a self-incompatable plant species. Claims 68 and 69 of record are amended to specify that the repressible lethal gene and repressor gene are located so that they segregate independently.

Claim 86 of record is amended to clarify that oncogenes 1 and 2 are of *Agrobacterium*. Claim 88 of record is cancelled, and the following claims renumbered as claims 87-97.

Applicants respectfully submit that the amendments to the description, claims and sequence listing do not constitute new matter.

Concerning Article 33(2) PCT

Claims 1, 5-7, 10, 28, 34, 39, 58, 60, 69, 70 and 97-99 of record stand rejected as lacking novelty under Article 33(2) PCT. The International Preliminary Examining Authority ("IPEA") contends that reference D1 pertains to a genetically modified plant comprising a first gene whose

expression results in a novel trait, a second gene encoding a recombinase, and a third gene encoding a repressor. The first gene is said to be linked to a promoter which is separated from the coding sequence by a blocking sequence, which again is flanked by excision sequences. The IPEA contends that specific embodiments are disclosed including the use of a lethal gene with a 35S promoter as the first gene.

Applicants respectfully traverse this rejection. Claim 1 of the instant application specifies, in step (c), "selecting a plant wherein the inserted repressor gene segregates independently from the inserted repressible lethal gene." Applicants respectfully submit that D1 does not teach or suggest this step, and that the subject matter of claim 1 is therefore novel over D1. Claims 5-7, 10, 28, 34, 39, 58 and 60 of record depend either from claim 1, or claim 14, or both. Claim 14 also includes a step of selecting a plant wherein the inserted repressor genes segregates independently from the inserted repressible lethal gene. Applicants respectfully submit that these further claims are therefore also necessarily novel over D1.

Claims 69 and 70 of record (new claims 68 and 69) are amended to specify that the repressible lethal gene and the repressor gene are located so that they segregate independently. Applicants respectfully submit that D1 does not disclose or suggest vectors having this characteristic as presently claimed.

Claims 97 and 98 of record (presently amended claims 95 and 96) stand rejected as being anticipated by D1, D2 and D3.

Applicants respectfully traverse this rejection. The DNA constructs of D1 have no utility for production of hybrid seed as described in the present application. Hence, D1 does not teach or suggest a plant transformation vector "for use in generating a parent plant for a hybrid cross" as claimed in instant claims 95 and 96. Applicants further respectfully submit that D2 and D3 do not disclose the use of a repressible lethal gene for hybrid seed production and do not teach or suggest plant transformation vectors for use in generating a parent plant for a hybrid cross, as claimed.

Claim 99 of record (claim 97 as presently amended) stands rejected as failing to contain any technical information and therefore being subject to the same arguments concerning novelty as

claims 97 and 98 of record. Presently amended claim 97 is directed to plasmids pG1, pG14, pPHAStet1 and pGG-14. It will be apparent to those of ordinary skill in the art that pG1, pG14, pPHAStet1 and pGG-14 are designators for particular plasmids, the construction of which is fully described in the examples and drawings of the instant application. Hence, the plasmids of instant claim 97 incorporate the technical features of such plasmids specified in the description. Applicants therefore respectfully submit that the claimed plasmids contain technical features distinguishing from references D1 - D3.

Concerning Article 33(3) PCT

Claims 2-4, 8, 9, 11-13, 23-27, 36-38, 61-68, 75-89 and 91-96 of record stand rejected under Article 33(3) PCT as lacking an inventive step in view of D1. The IPEA acknowledges that D2 and D3 do not disclose subject matter going beyond the disclosure of D1, and therefore have not been applied in this rejection.

Applicants respectfully traverse this rejection. All of claims 2-4, 8, 9, 11-13, 23-27, 36-38, and 61-68 of record are ultimately dependent on one of claims 1, 14, 40, 43, 44, or 53. Claims 1, 14, 40, and 44 of record each specifies that the inserted repressor gene segregates independently from the inserted repressible lethal gene, a limitation that is not taught or suggested by D1. Similarily D2 and D3 do not teach or suggest independent segregation of a repressible lethal gene and a repressor gene. Claims 43 and 53 of record do not stand rejected. Applicants therefore respectfully submit that claims 2-4, 8, 9, 11-13, 23-27, 36-38, and 61-68 of record are inventive over the teachings of D1-D3.

With respect to claims 75-89 and 91-96 of record, when they are dependent on claim 69 or 70 of record, claims 69 and 70 (now claims 68 and 69) are amended to specify that the repressible lethal gene and the repressor gene are located so that they segregate independently, a limitation that is not taught or suggested by any of D1 – D3, as discussed above, and are therefore novel and inventive over D1 – D3. Claim 75 of record specifies that the vector is "for use in generating a parent plant for a hybrid cross". As discussed earlier, the DNA constructs of D1 have no utility for production of hybrid seed as described in the present application. Hence, D1 does not teach or suggest a plant transformation vector as claimed in claim 75 of record (now claim 74). Applicants

further respectfully submit that D2 and D3 do not disclose the use of a repressible lethal gene for hybrid seed production and do not teach or suggest plant transformation vectors for use in generating a parent plant for a hybrid cross, as claimed. Therefore, Applicants respectfully submit that, claims 75-89 and 91-96 of record, when they are dependent on claim 75, are necessarily novel and inventive over D1-D3.

Concerning Item VII

Pages 66, 68, 70 and 71 of record are objected to for incorrect references to sequences in bold face or underlined font. Pages 66, 68, 70 and 71 are presently amended to depict the sequences in bold face and underlined font, conforming with the related description.

An objection is raised to SEQ ID NO: 8 of the sequence listing as not being identical with SEQ ID NO: 8 in the description on page 68. Accordingly, SEQ ID NO: 8 in the sequence listing is presently amended to conform with SEQ ID NO: 8 at page 68.

Concerning Item VIII

The IPEA contends that claims 10 and 86 of record are unclear on the grounds that no source of oncogenes 1 and 2 is indicated. Claims 10 and 86 (now claim 85) are presently amended to clarify that oncogenes 1 and 2 are oncogenes 1 and 2 of *Agrobacterium*.

Claim 25 of record is objected to on the basis that, in the case that only one of the lethal genes is selected from the specified group, it is unclear what the other gene is or where it is selected from. Applicants note that claim 25 is dependent on claim 14. Claim 14 does not specify the source of the repressible lethal genes, and has not been objected to as lacking clarity only for this reason. Claim 25 adds the further requirement that at least one of the repressible lethal genes specified in claim 14 is selected from a specified group. Applicants respectfully submit that, as claim 25 adds a further limitation to claim 14, which in turn is recognized by the IPEA as being clear, claim 25 necessarily also meets the requirements of clarity pursuant to Article 6 PCT.

Claims 27 and 88 of record are objected to as being redundant over other pending claims. Claims 27 and 88 of record are cancelled in the present amendments.

The IPEA objects to the terms pG1, pG14, pPHAStet1 and pGG-14 in claim 99 of record as being private denominations which are meaningless to the public. Applicants respectfully traverse this objection and submit that these terms are not private denominations which are meaningless to the public. Rather, the terms pG1, pG14, pPHAStet1 and pGG-14 are designations of particular plasmids, the details of which are provided in the examples and drawings of the instant application. The designations are not private in that their meaning is clear and apparent on the face of the application. Those of ordinary skill in the art will understand that the terms pG1, pG14, pPHAStet1 and pGG-14 identify particular plasmids fully described in the examples and drawings by their particular technical features.

Claims 42, 46, 55 and 58 of record are objected to as being in the form of product by process claims. Applicants will address issues of claim construction and patentability during the national phase.

In view of the foregoing, entry of the amendments and reconsideration and withdrawal of the rejections of the claims are respectfully requested. Early issuance of an International Preliminary Examination Report that is positive as to the novelty, inventive step and industrial applicability of all of the pending claims is earnestly solicited.

Yours very truly,

Ottawa, Canada

SMART & BIGGAR

Encl.

(Mrs.) Joy D. Morrow

March 21, 2001

constructs are to be transferred to sexually incompatible relatives tissue culture techniques such as wide crosses and/or embryo rescue may be employed. A variety of techniques known to those skilled in the art may be employed to derive the combination of repressible lethal and repressor genes which provides the greatest utility within the scope of the present invention.

The following examples are set forth to illustrate the method and in no way limit the scope of the invention.

Example 1: Isolation of oncogene 1 and 2 from *Agrobacterium* Ti-plasmid pTi15955.

To isolate the oncogenes, the following steps were employed. The subclones p101 and p202, detailed in US 5,428,147 encompassing the DNA encoding oncogene 1 (p202) and oncogene 2 (p101) are used as a source of the genes. In order to isolate the genes, a combination of PCR to introduce convenient restriction sites and subcloning of native gene fragments is employed to derive oncogenes that can be conveniently inserted into plant transformation vectors.

To isolate a native oncogene 2, the following approach is used. The 5' region, including the native promoter of oncogene 2 is isolated by PCR amplification of the plasmid p101 with the following primers:

G2P1 (SEQ ID NO.1)

5'ATA**GCATGC**TCTAGATGTTAGAAAAGATTCGTTTTTGTG 3'

and, G2P2 (SEQ ID NO:2)

5' ATA**CCATGG**CGATCAATTTTTTTGGCGC 3'

G2P1 contains a Sph 1 site (boldface) and a Xba I site (underlined) and corresponds to the complement of nucleotides 5808 - 5785 in the published sequence of pTi15955. G2P2 contains a Nco 1 site (boldface) and corresponds to nucleotides 5285 - 5309 in the published sequence of pTi15955. The use of G2P1 and G2P2 yields a fragment of 523 bp which represents the 5' region of the native oncogene 2, including the promoter modified to contain a Sph 1 and Xba I site at the 5' end of the promoter.

To isolate the 3' region of oncogene 2, including the native terminator structure, two PCR primers are used. The first primer used is:

G2P3 (SEQ ID NO:3)

5' ATA**AAGCTT**GAAAATTAAGCCCCCCCCCG 3'

and, G2P4 (SEQ ID NO:4)

5' ATAGGATCC**GCATGCC**CAGTCTAGGTCGAGGGAGGCC 3'

G2P3 contains a Hind III site (boldface) and corresponds to the complement of nucleotides 3396 - 3371 of the published sequence of pTi 15955. G2P4 contains a Sph 1 site (boldface) and a Bam H1 site (underlined) and corresponds to nucleotides 3237 - 3264 of the published sequence of pTi 15955. The use of G2P3 and G2P4 yields a fragment of 164 bp which represents a portion of the 3' end of the native oncogene 2.

The plasmid p101 is digested with Nco I and Hind III to yield a fragment of approximately 1895 bp fragment of

oncogene 2 which encompasses most of the coding region. The 523 bp fragment of the 5' end of the native oncogene 2 is digested with Nco I and ligated to the Nco I site of the 1895 bp fragment and the 164 bp 3' end of the gene is digested with Hind III and ligated to the Hind III site of the 1895 bp fragment. The reconstructed native oncogene 2 is then digested with Sph 1 and subcloned into the Sph 1 site of the common cloning vector pGEM-4Z (Promega, La Jolla, California). This vector is called pG2. DNA sequencing was used to verify the composition of this reconstructed DNA corresponding to the authentic DNA sequence of the native oncogene 2.

Isolation of oncogene 1 employs a combination of PCR to introduce convenient restriction sites and subcloning of a native gene fragment. To isolate the required fragments, the following approach is used. Convenient restriction sites at the 5' end of the coding region are introduced by PCR, employing the following two primers:

G1P1 (SEQ ID NO:5)

5' ATA**ATCGATAT**AGAAACGGTTGTTGTGGTT 3'

and, G1P2 (SEQ ID NO:6)

5' ATA**AGATCT**CGGGGAAGCGACC 3'

G1P1 contains a Cla 1 site (boldface) and corresponds to nucleotides 5755 - 5775 of the published sequence of pTi 15955. G1P2 contains a Bgl II site (boldface) and corresponds to the complement of nucleotides 6028 - 6010 of the published sequence of pTi 15955. G1P1 and G1P2 are used to amplify a 273 bp fragment of oncogene 1 which is modified to contain a Cla 1 site at the 5' end of the coding region.

To isolate a 3' fragment of the coding region of oncogene 1, two primers are used to introduce convenient restrictions sites at the 3' end of the coding region.

G1P3 (SEQ ID NO:7)

5' AAT**GATATC**TGAACTTTATGATAAGG 3'

and, G1P4 (SEQ ID NO:8)

5' ATAGAGCTC**ATCGAT**ACTAATTTCTAGTGCGGTAGTT 3'

G1P3 contains a Eco RV site (boldface) and corresponds to nucleotides 7350 - 7372 of the published sequence of pTi 15955. G1P4 contains a Cla 1 site (boldface) and a Sac 1 site (underlined) and corresponds to nucleotides 8076 - 8056 of the published sequence of pTi 15955. The use of G1P3 and G1P4 results in a 732 bp fragment representing the 3' end of the coding region of oncogene 1.

In order to reconstruct a complete coding region of the oncogene 1, the plasmid p202 is digested with Bgl II and the 1697 bp fragment encompassing the partial coding region of the oncogene 1 is isolated. To the 5' end of

containing three copies of the tet operator sequence. The means by which this is accomplished is as follows and is shown in Figure 8.

The promoter region of the phaseolin gene (described in: Slightom, J.L., Sun, S.M. and Hall, T.C., Proc. Natl. Acad. Sci. USA 80:1897-1901, 1983) is isolated by PCR using the vector pAGM 219, kindly supplied by Dr. G. Cardineau of Mycogen Plant Sciences, San Diego, California. The plasmid pAGM 219 contains approximately 1600 base pairs of the promoter region of the phaseolin gene and the native termination region of the phaseolin gene. The region of the promoter 5' to the TATA box was isolated by PCR in preparation for the addition of a synthetic DNA sequence comprising the tet operator DNA and a TATA box.

The first PCR primer used was engineered to introduce a Csp45 1 site by a minor alteration of the nucleotide sequence in the native promoter sequence. The sequence of this primer is shown below:

SEQ ID NO:9

5'GGTGG**TTCGAA**CATGCATGGAGATTTG 3'

The Csp45 1 restriction site is shown in boldface. The second primer used for PCR has the following sequence:

SEQ ID NO:10

5'CCGTAT**CTCGAG**ACACATCTTCTAAAGTAATTT 3'

A Xho 1 site is indicated in boldface. The PCR product obtained using these primers was called pPHAS and corresponds to nucleotides 128 - 833 of the DNA sequence of the phaseolin promoter of the lambda genomic clone AG-λPVPh177.4 (λ177.4), (Slightom, J.L., Sun, S.M. and Hall, T.C., Proc. Natl. Acad. Sci. USA 80:1897-1901, 1983). A synthetic tet operator sequence was added to this fragment by joining the synthetic duplex DNA to the Csp45 1 site in the PCR product. The synthetic operator DNA sequence also comprises a Cla 1 site at the 3′ end of the sequence. The top strand of the synthetic DNA has the following sequence:

SEQ ID NO:11

5'*TTCGAA*GACTCTATCAGTGATAGAGTGTATATAAGACTCTATCAGTGATAG AGTGAACTCTATCAGTGATACAGTAT*ATCGAT* 3'

Which comprises 3 copies of the operator DNA (boldface), a TATA box (underlined), a Csp45 1 site at the 5′ end (italics and underlined) and a Cla 1 site at the 3′ end (italics and boldface). A bottom strand fragment is used which has the following sequence:

SEQ ID NO:12

5'**CG**ATATACTGTATCACTGATAGAGTTCACTCTATCACTGATAGAGTCTTAT ATACACTCTATCACTGATAGAGTCTTCGTT 3'

Which comprises a complementary strand to SEQ ID NO:9 and contains a Cla 1 cohesive end, identified in boldface. The duplex DNA is referred to a "top" DNA and is ligated to the Csp45 1 and Cla 1 cut pPHAS and clones containing the inserted "top" DNA are chosen. This vector

12. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene is separate from the DNA construct comprising the repressor gene, and wherein the constructs are introduced simultaneously into a plant cell capable of being transformed and regenerated into a whole plant.

13. The method of claim 1 wherein the DNA construct comprising the repressible lethal gene is the same as the DNA construct comprising the repressor gene, said DNA construct further comprising a DNA sequence that allows independent segregation of the inserted repressible lethal gene and the inserted repressor gene.

14. A method of making a genetically modified plant comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:

(i) a DNA construct comprising a first repressible lethal gene, the activity of whose gene product is lethal to plant cells;

(ii) a DNA construct comprising a first repressor gene whose gene product is capable of repressing said first lethal gene activity;

(iii) a DNA construct comprising a second repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(iv) a DNA construct comprising a second repressor gene whose gene product is capable of repressing said second lethal gene activity;

(b) regenerating from said plant cell a whole plant; and,

(c) selecting a plant wherein the inserted first repressor gene segregates independently from the inserted first repressible gene, and wherein the inserted second repressor gene segregates independently from the inserted second repressible gene.

15. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is separate from the DNA construct comprising the first repressor gene; wherein the DNA construct comprising the second repressible lethal gene is separate from the DNA construct comprising the second repressor gene; and wherein selection is accomplished by crossing the plants generated in step (b).

16. The method of claim 14 wherein the inserted first repressible lethal gene and the inserted first repressor gene are located on opposite sister chromosomes of a chromosome pair, and wherein the inserted second repressible lethal gene and the inserted second repressor gene are located on opposite sister chromosomes of a chromosome pair.

17. The method of claim 14 wherein the first repressible lethal gene is within the same DNA construct as the first repressor gene, and wherein the second repressible lethal gene is within the same DNA construct as the second repressor gene; and wherein independent segregation is effected by use of a site-specific recombinase or a transposase.

18. The method of claim 14 wherein the first repressible lethal gene is linked to the second repressor gene, and wherein the second repressible lethal gene is linked to the first repressor gene.

19. The method of claim 14 wherein the first or second or both repressible lethal gene is under control of a tissue-specific promoter.

20. The method of claim 14 wherein:

(a) the DNA construct comprising the second repressible lethal gene also comprises a first specific DNA operator sequence, wherein the first repressor is a bacterial repressor capable of binding to the first specific DNA operator sequence, and wherein the binding between the first bacterial repressor and the first specific DNA operator sequence results in repression of the second lethal gene; and,

(b) the DNA construct comprising the first repressible lethal gene also comprises a second specific DNA operator sequence, wherein the second repressor is a bacterial repressor capable of binding to the second specific DNA operator sequence, and wherein the binding between the second bacterial repressor and the second specific DNA operator sequence results in repression of the first lethal gene.

21. The method of claim 14 wherein the first or second or both repressor gene is under control of a tissue-specific promoter.

22. The method of claim 14 wherein the first, or first and second, repressor gene is under control of a constitutive promoter.

23. The method of claim 1 or 14 wherein the repressor comprises an antisense RNA or a sense gene capable of inhibiting expression of the repressible lethal gene.

24. The method of claim 1 or 14 wherein the repressor comprises a ribozyme capable of inhibiting expression of the repressible lethal gene.

25. The method of claim 14 wherein either or both repressible lethal genes is selected from the group consisting of: oncogenes 1 and 2 of *Agrobacterium*, oncogenes 1 and 2 of *Pseudomonas*, oncogene 4 of *Agrobacterium*, a gene encoding a ribosome inactivating protein, a gene encoding diptheria A chain toxin, and a gene encoding a ribonuclease.

26. The method of claim 10 or 25 wherein oncogenes 1 and 2 are from *Agrobacterium* or *Pseudomonas*.

27. The method of claim 10 or 25 wherein the ribonuclease is Barnase or T1 ribonuclease.

28. The method of claim 14 wherein the DNA construct comprising a first repressible lethal gene, or the DNA construct comprising a second repressible lethal gene, or both, further comprises a conditionally lethal gene.

29. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is separate from the DNA construct comprising the first repressor gene; wherein the DNA construct comprising the second repressible lethal gene is separate from the DNA construct comprising the second repressor gene, and wherein the constructs are introduced simultaneously into a plant cell capable of being transformed and regenerated into a whole plant.

30. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is the same as the DNA construct comprising the first repressor gene; wherein the DNA construct comprising the second repressible lethal gene is

the same as the DNA construct comprising the second repressor gene, said DNA constructs further comprising a DNA sequence that allows independent segregation of the inserted first repressible lethal gene from the inserted first repressor gene, and that allows independent segregation of the inserted second repressible lethal gene from the inserted second repressor gene.

31. The method of claim 14 wherein the DNA construct comprising the first repressible lethal gene is the same as the DNA construct comprising the second repressor gene; wherein the DNA construct comprising the second repressible lethal gene is the same as the DNA construct comprising the first repressor gene, said DNA constructs being contained within a single plant transformation vector that allows independent segregation of the DNA constructs.

32. The method of claim 31 wherein independent segregation is effected by use of a site-specific recombinase or a transposase.

33. The method of any one of claims 5, 19 and 21 wherein the tissue specific promoter is a seed specific promoter.

34. The method of claim 33 wherein the seed specific promoter is the phaseolin promoter.

35. The method of claim 1 or 14 wherein the plant further comprises a novel trait.

36. The method of claim 35, wherein the novel trait comprises an altered phenotype, a protein not found in the native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.

37. The method of claim 35 or 36, wherein the gene encoding the novel trait is linked to a repressible lethal gene.

38. The method of any one of claims 35 to 37, wherein the gene encoding the novel trait is under the control of a seed specific promoter.

39. A method of producing, for a hybrid cross, a male parent comprising a repressible lethal gene that is expressed during outcrossing or introgression of alien germplasm, comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:

(i) a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(ii) a DNA construct comprising a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an inducible promoter;

wherein the inserted repressor gene segregates independently from the inserted repressible gene;

(b) regenerating from said plant cell a whole plant;

(c) generating from said plant, a plant which is homozygous for the repressible lethal gene and inducible repressor gene;

wherein the homozygous plant can function as the male parent for a hybrid cross.

40. A method of maintaining the male parent of claim 39, comprising:

(a) permitting the homozygous plant to self-pollinate;

(b) inducing the inducible promoter in the homozygous plant during the period of time that seed formation takes place; and

(c) obtaining the seed.

41. A plant produced by the method of claim 39 or 40

42. A method of producing, for a hybrid cross, a female parent that carries a repressible lethal gene, comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(b) regenerating from said plant cell a whole plant.

43. A method of producing, for a hybrid cross, a female parent comprising a repressible lethal gene and an inducible repressor gene, comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant:

(i) a DNA construct comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(ii) a DNA construct comprising a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an inducible promoter;

wherein the inserted repressor gene segregates independently from the inserted repressible gene;

(b) regenerating from said plant cell a whole plant;

(c) generating from said plant, a plant which is homozygous for the repressible lethal gene and inducible repressor gene;

wherein the homozygous plant can function as the female parent for a hybrid cross.

44. A method of maintaining the female parent of claim 43 comprising:

(a) permitting the homozygous plant to self-pollinate;

(b) inducing the inducible promoter in the homozygous plant during the period of time that seed formation takes place; and

(c) obtaining the seed.

45. A plant produced by the method of any one of claims 42 to 44.

46. The method of any one of claims 39, 40, 42 to 44 wherein said seed specific promoter comprises the phaseolin promoter.

47. The method of any one of claims 39, 40, 42 to 44 wherein the plant further comprises a novel trait.

48. The method of claim 47, wherein the novel trait comprises an altered phenotype, a protein not found in the native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.

49. The method of claim 47 or 48, wherein the gene encoding the novel trait is linked to a repressible lethal gene.

50. The method of any one of claims 47 to 49, wherein the gene encoding the novel trait is under the control of a seed specific promoter.

51. A method of producing hybrid seed comprising a repressible lethal gene and a repressor gene, comprising:

(a) allowing the plant of claim 45 to flower;

(b) fertilizing said plant with pollen which comprises a repressor gene whose gene product is capable of repressing said repressible lethal gene; and,

(c) recovering hybrid seed which comprises a repressible lethal gene and a repressor gene.

52. A method of producing, for a hybrid cross, a female parent that carries a repressor gene, comprising:

(a) introducing into a plant cell capable of being transformed and regenerated to a whole plant a DNA construct comprising a repressor gene;

(b) regenerating from said plant cell a whole plant;

(c) generating from said plant a plant which is homozygous for the repressor gene;

wherein the homozygous plant can function as the female parent for a hybrid cross.

53. The method of claim 52 wherein the female parent is incapable of self-pollination.

54. A plant produced by the method of claim 52 or 53.

55. A method of making a genetically modified plant seed comprising a repressible lethal gene that is expressed during outcrossing or introgression of alien germplasm, comprising:

(a) crossing the male parent of claim 41 with the female parent of claim 54, wherein the repressor gene product of the female parent is capable of repressing the repressible lethal gene of the male parent;

(b) harvesting hybrid seed from the male parent, comprising the repressible lethal gene from the male parent and the repressor gene from the female parent.

56. The method of claim 55 wherein the plant seed is derived from a self-incompatible plant species.

57. A plant produced by the method of any one of claims 1 to 38, 51, 55 and 56.

58. The plant of any one of claims 41, 45, 54 and 57, further comprising a marker gene.

59. The plant of claim 57 or 58, which is a dicot (*Dicotyledoneae*).

60. The plant of claim 57 or 58, which is a monocot (*Monocotyledoneae*).

61. The plant of claim 57 or 58, which is a member of the family: *Malvaceae, Linaceae, Compositae, Fabaceae, Euphorbiaceae,* or *Oleaceae.*

62. The plant of claim 57 or 58, which is a member of the genus: *Brassica.*

63. The plant of claim 57 or 58, which is a member of the genus: *Linum, Gossypium, Glycine, Arachis, Carthamus, Helianthus, Sinapis, Raphanus, Ricinus* or *Olea.*

64. The plant of claim 57 or 58, which is a member of the family *Brassicaceae (= Cruciferae).*

65. The plant of claim 57 or 58, which is a *Brassica napus* or *rapa.*

66. The plant of claim 57 or 58, which is a a rapeseed or canola.

67. The plant of claim 57 or 58, which is an oilseed plant.

68. A plant transformation vector, for use in limiting outcrossing of a plant transformed with said vector, comprising:

(a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(b) a repressor gene whose gene product is capable of repressing the activity of said lethal gene product; said repressible lethal gene and said repressor gene being located so that they segregate independently.

69. A plant transformation vector, for use in limiting introgression of alien germplasm, comprising:

(a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(b) a repressor gene whose gene product is capable of repressing the activity of said lethal gene product; said repressible lethal gene and said repressor gene being located so that they segregate independently.

70. A plant transformation vector, for use in limiting outcrossing of a plant transformed with said vector or for limiting introgression of alien germplasm, comprising:

(a) a first repressible lethal gene, the activity of whose gene product is lethal to plant cells;

(b) a first repressor gene whose gene product is capable of repressing said first lethal gene activity;

(c) a second repressible lethal gene, the activity of whose gene product is lethal to plant cells; and,

(d) a second repressor gene whose gene product is capable of repressing said second lethal gene activity.

71. The vector of claim 70, wherein the first repressible lethal gene is not linked to the first repressor gene, and wherein the second repressible lethal gene is not linked to the second repressor gene.

72. The vector of claim 70, wherein the first repressible lethal gene is linked to the first repressor gene, and wherein the second repressible lethal gene is linked to the second repressor gene, and wherein the vector further comprises at least one DNA sequence that allows independent segregation of the first repressible lethal gene from the first repressor gene, and independent segregation of the second repressible

lethal gene from the second repressor gene, after transformation.

73. The vector of any one of claims 70 to 72, wherein:

(a) a first specific DNA operator sequence is linked to the second repressible lethal gene, wherein the first repressor is a bacterial repressor capable of binding to the first specific DNA operator sequence, and wherein the binding between the first bacterial repressor and the first specific DNA operator sequence results in repression of the second lethal gene; and,

(b) a second specific DNA operator sequence is linked to the first repressible lethal gene, wherein the second repressor is a second bacterial repressor capable of binding to the second specific DNA operator sequence, and wherein the binding between the second bacterial repressor and the second specific DNA operator sequence results in repression of the first lethal gene.

74. A plant transformation vector, for use in generating a parent plant for a hybrid cross, comprising:

(a) a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter; and,

(b) a repressor gene, whose gene product is capable of repressing said lethal gene activity, under control of an inducible promoter.

75. The vector of any one of claims 68, 69 and 74, wherein the repressible lethal gene and the repressor gene are not linked.

76. The vector of any one of claims 68, 69 and 74, wherein the repressible lethal gene and the repressor gene are linked, and wherein the vector further comprises at least one DNA sequence that allows independent segregation of the repressible lethal gene and the repressor gene after transformation.

77. The vector of claim 72 or 76, wherein the at least one DNA sequence comprises a recognition sequence for a site-specific recombinase or a transposase.

78. The vector of any one of claims 68 to 77, wherein the repressible lethal gene or the repressor gene or both is under control of a tissue-specific promoter.

79. The vector of claim 78 wherein the tissue-specific promoter is a seed specific promoter.

80. The vector of claim 79 wherein the seed specific promoter is the phaseolin promoter.

81. The vector of any one of claims 68, 69 and 74 to 77, wherein a specific DNA operator sequence is linked to the repressible lethal gene, wherein the repressor is a bacterial repressor capable of binding to the specific DNA operator sequence, and wherein the binding between the bacterial repressor and the specific DNA operator sequence results in repression of the lethal gene.

82. The vector of any one of claims 68 to 77, wherein the repressor gene is under control of a constitutive promoter.

83. The vector of any one of claims 68 to 77, wherein the repressor gene is under control of an inducible promoter.

84. The vector of any one of claims 68 to 77, wherein the activity of the lethal gene product comprises over-expression or under-expression of a naturally occurring plant growth regulating substance.

85. The vector of any one of claims 68 to 77, wherein the repressible lethal gene is selected from the group consisting of: oncogenes 1 and 2 of *Agrobacterium*, oncogene 4 of *Agrobacterium*, gene encoding a ribosome inactivating protein, gene encoding diptheria A chain toxin, and a gene encoding a ribonuclease.

86. The vector of claim 85 wherein oncogenes 1 and 2 are from *Agrobacterium* or *Pseudomonas*.

87. The vector of claim 85 wherein the ribonuclease is Barnase or T1 ribonuclease.

88. The vector of any one of claims 68 to 77, further comprising a conditionally lethal gene linked to the repressible lethal gene.

89. The vector of any one of claims 68 to 77, wherein the repressor gene is under control of a constitutive promoter.

90. The vector of any one of claims 68 to 77, wherein the product of the repressor gene comprises an antisense RNA or a sense gene or a ribozyme capable of inhibiting expression of the repressible lethal gene.

91. The vector of any one of claims 68 to 77, further comprising a gene encoding a novel trait.

92. The vector of claim 91, wherein the novel trait comprises an altered phenotype, a protein not found in the

native plant cell, or a protein not found in the native plant cell and which confers no detectable phenotype.

93. The vector of claim 91 or 92, wherein the gene encoding the novel trait is linked to the repressible lethal gene.

94. The vector of any one of claims 91 to 93, wherein the gene encoding the novel trait is under the control of a seed specific promoter.

95. A plant transformation vector, for use in generating a parent plant for a hybrid cross, comprising a repressible lethal gene, the activity of whose gene product is lethal to plant cells, under control of a seed specific promoter.

96. A plant transformation vector, for use in generating a parent plant for a hybrid cross, comprising a repressor gene.

97. Plasmid pG1, pG14, pPHAStet1, pGG-14.